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Rheological Characterization of Agarose and Poloxamer 407 (P407) Based Hydrogels

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ABSTRACT

Poloxamer 407 (P407) is a biocompatible thermo-setting polymer, while agarose is a biocompatible thermo-softening material. It is interesting to mix them to examine any possible synergy in thermomechanical properties. In this study, rotational rheometer was adopted to study rheological properties of the mixtures of agarose/P407 gels with different concentrations at various frequencies, strain rates and temperatures. It has revealed that the addition of P407 decreased the gel stiffness by an order of magnitude. For the given combinations in this study, the increase in agarose concentration would increase both the storage modulus and loss modulus of the gel mixtures. The variation in P407 concentration (2.5%-10%) minimally changes the composite moduli. These agarose/P407 gel mixtures also exhibited shear thinning behavior. However, the addition of P407 (2.5%-10%) to agarose gel only has very small effect on thermomechanical properties of agarose gels. The overall transition temperature for these gel mixtures is governed by P407 melting point where the phase change starts around 55°C and the gels completely collapse at the melting temperature of agarose.

INTRODUCTION

Hydrogels have been widely used in biomedical engineering applications[1-4]. These highly hydrated polymeric networks enable cell encapsulation due to their resemblance to extracellular matrix and remarkable biocompatibility[5-10]. For example, agarose gel has been used as a tissue filler for skin[11] and as cell capsules for tumor therapy[12]. When agarose is melted in water, thermo-reversible gels with appropriate properties can be formed and their mechanical properties can be easily adjusted by changing the concentration of the polymer[13-15]. Therefore, they have been used as scaffold materials which enable cells to maintain their phenotypes[16]. Poloxamer 407 (P407) has been commonly used to optimize drug formulation due to its thermo-reversible properties[17]. It presents a liquid like behavior below sol-gel transition temperature to provide an exact delivery, and it transforms into gel at higher temperatures[18]. Their sol-gel transition can be controlled by the polymer concentration and other additives[19]. A rheological study shows that P407 gels are viscoelastic materials and their viscosity decreases during shear deformation since they are pseudo plastic[20]. *In*

vitro tests released that an increase in P407 concentration resulted in greater gel strength which may be altered by additives or drugs[17].

It is interesting to examine the possible synergy in thermomechanical properties by mixing agarose (thermo-softening) and P407 (thermo-setting) to form a composite gel. However, there is a lack of comprehensive studies to investigate the rheological properties of agarose/P407 mixtures which can affect their potential applications. Therefore, this study aimed to synthesize various agarose/P407 gels and investigate their rheological properties at different conditions.

EXPERIMENTAL DETAILS

Materials and sample preparation

Purified and non-ionic P407 powder and Agarose Type VII-A powder with low gelling temperature were purchased from Sigma-Aldrich Co. P407 gels are usually prepared at a concentration of 20-30% (w/v)[17] , however, homogenous agarose/P407 mixtures was not achievable when P407 concentration (w/v) exceeds 10%. Therefore, after homogeneity of gel mixtures were optimized, the following samples (with all the concentrations in w/v) were prepared: 1.25% agarose with 2.5% or 5% P407; 2.5% agarose with 2.5%, 5% or 10% P407; 5% agarose with 2.5% P407, and 5% agarose with 5% P407. Both agarose and P407 powder were mixed with deionized water, which was autoclaved at 121ºC and 1 bar for 15 minutes. The gel solution was injected in a mold holding 8 samples, each having a diameter of 20mm and a thickness of 3mm. The mold was very carefully handled between two glasses to enable uniform thickness and good surface finishing of the samples. The samples were kept in deionized water prior to testing.

Mechanical testing

Rheological characterization of hydrogels was carried out using a Malvern Kinexus Pro+ rotational rheometer (Worcestershire, UK). This rheometer was equipped with a solvent trap system to prevent dehydration of the hydrogels during the tests. Serrated parallel plates with a diameter of 20mm were used to avoid slippage. To identify linear viscoelastic region (LVR), oscillatory strain sweep tests were applied between the strain values of 0.01% and 10%. Frequency sweep tests were then applied between 0.1 Hz and 10 Hz at a strain value within the LVR (0.05% in this study) to obtain the storage modulus (G'), loss modulus (G") and complex shear viscosity (η*). For shear rate sweep tests, shear rate value varied between $0.1s^{-1}$ and $100s^{-1}$ to examine the possible shear thinning behavior. All those rheological tests were carried out at 25ºC. Thermal behavior characterization was done by temperature sweep tests and the tested temperature range was 2ºC–70ºC. For each test, at least three replicates were made. ANOVA test was used for statistical analysis (α=0.05).

RESULTS AND DISCUSSION

Mechanical properties of gel mixtures by frequency sweep tests

The dependency of storage moduli (G') and loss moduli (G'') on frequency is presented in Figure 1. For all tested gels, both shear moduli values were independent of frequency for the tested frequency range of 0.1Hz to 10Hz, implying that the mixtures

resembled elastic hydrogels[21]. Storage moduli values being significantly greater than viscous moduli values for all concentrations also denoted that agarose gels with P407 modification were elastically dominated[22]. The frequency independent behavior suggests that the gels were well mixed with stable microstructure. For cured gels, it was also found elsewhere that both G' and G" were stable during frequency sweep (0.01-100Hz) tests[23].

For gels with constant agarose concentration (Fig. 1a), both G’ and G’’ values did not follow a trend by varying P407 concentration. The gels with an increasing concentration of P407 (from 2.5% to 10%) had G’ values of 21.8kPa, 18.4 kPa and 26.7 kPa when the agarose concentration was kept constant at 2.5%. For gels with constant P407 concentration (Fig. 1b), both shear moduli values increased with the increasing concentration of agarose (storage moduli of 6.09 kPa, 21.8 kPa and 28.2 kPa) when the P407 concentration was kept constant at 2.5%. It was found that the storage moduli of 1%–5% agarose hydrogels were frequency independent and ranged between 38kPa to 400kPa at 35°C[24] and the storage modulus of 18% P407 at 35°C is nearly 0.1Pa and increases with the raised concentration of P407 and frequency[25, 26]. Therefore, the storage and loss moduli of agarose/P407 mixtures were dominated by the agarose content.

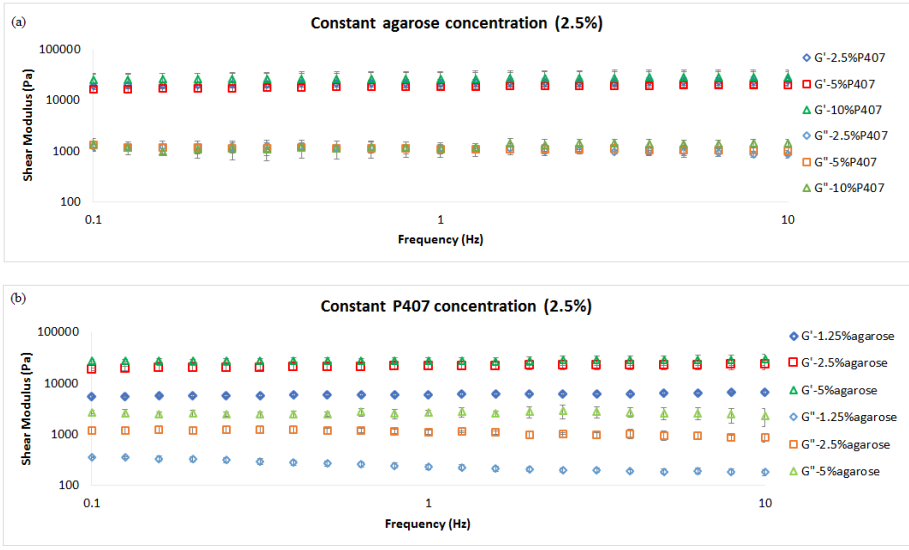

Figure 1. Frequency dependent behavior of storage (G’) and viscous (G”) shear moduli for gel mixtures for (a) 2.5% agarose mixed with varying concentrations of P407 and (b) 2.5% P407 mixed with varying concentrations of agarose

When the proportion of agarose was small (1.25% and 2.5%), storage and loss moduli did not change significantly when the P407 concentration increased from 2.5% to 5%. However, when the content of agarose was higher, both of the G’ and the G’’ values rose rapidly with increased P407 concentration. The storage modulus of 5% agarose with 5% P407 gel was nearly three times as the value of 5% agarose with 2.5% P407 gel, and the viscous modulus was increased by a factor of 4. This suggests that the P407 modification has a more significant effect on the gels with higher agarose content, but when the concentration of agarose was low (lower than 2.5%), the influence from P407 was insignificant. The presented results showed that the change in the concentration of P407 from 2.5–10% minimally changed the composite moduli.

Shear thinning analysis

Shear rate dependent behavior of agarose/P407 gel mixtures is shown in Figure 2. When shear rate increased, the viscosity of the gels decreased. The change of viscosity with shear rate was not significantly different ($p>0.05$) among the gels. The trend of the viscosity change was the same among all tested hydrogels for both tested variables which can be attributed to the hindrance and friction among the different polymer chains which was also observed in different type of hydrogel mixtures[27]. This type of behavior, *i.e.* when viscosity significantly decreases with increasing shear rate, is referred as shear thinning behavior which suggests that the gel mixture of agarose and P407 can be injected by applying high shear rate during injection and also they show self-heal characteristics shortly after removing shear stress[28]. Agarose hydrogels in our rheological tests also presented shear thinning behavior (data not shown). A study[29] revealed that P407 gels did not show significant decrease between the shear rate of 1–1000 s^{-1}. Therefore, the shear thinning behavior is governed by agarose.

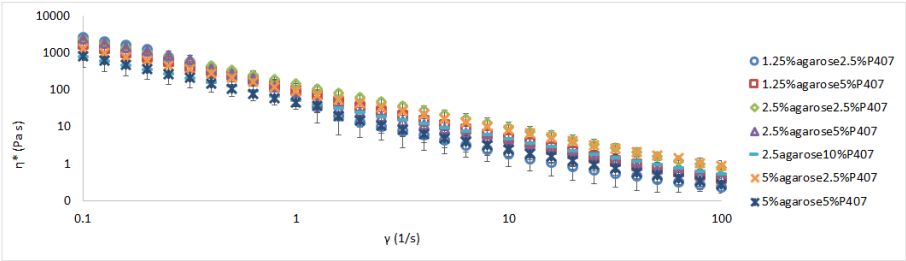

Figure 2. Change of complex viscosity of gel mixtures with shear rate

Temperature dependent behavior

The dependency of storage moduli (G') and loss moduli (G'') on temperature is presented in Figure 3. A near-constant value for G'' was observed for the temperature range of 2°C to ~55°C when 2.5% agarose was mixed with varying concentrations of P407 (Fig. 3a) and when 2.5% P407 was mixed with varying concentrations of agarose (Fig. 3b). G' values gradually decreased among this range of temperature for gel mixtures especially with higher P407 concentration (Fig. 3a) and higher agarose concentration (Fig. 3b), however this decrease was not significant. The upper temperature limit of ~55°C increased slightly by increasing agarose content. After melting initiated, the gel started to decompose into a liquid form regardless of the P407 content even though this type of gel shows thermosetting behavior, *i.e.* it stays solid-like at higher temperatures (>10°C) and liquid-like at low temperatures (~4°C).

Using a differential scanning calorimeter (DSC), the melting point of P407 gels at higher temperature cycles was found to be 55°C[30]. Agarose gel melting temperature was obtained as ~65°C by temperature sweep tests (data not shown). This suggests that the P407 modification has an effect on the melting point and overall melting point of the agarose and P407 gel mixtures were governed firstly by P407 which has the lower melting temperature.

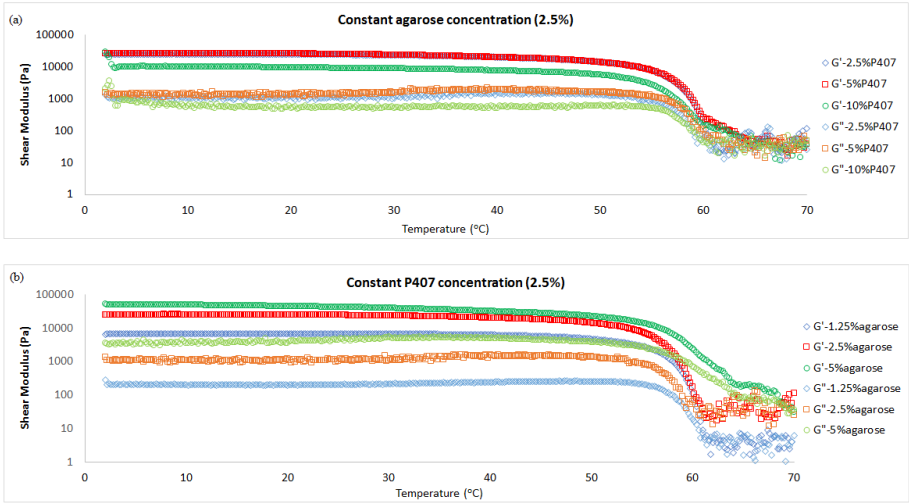

Figure 3. Temperature dependent behavior of storage (G') and viscous (G") shear moduli for gel mixtures for (a) 2.5% agarose mixed with varying concentrations of P407 and (b) 2.5% P407 mixed with varying concentrations of agarose (Error bars removed for visualisation.)

CONCLUSIONS

The rheological tests have revealed that the agarose/P407 gel mixtures were elastically dominated. The addition of P407 reduced the agarose gel stiffness by an order of magnitude. For the given combinations in this study, the increase of the concentration of agarose led to a rise in both the storage modulus and loss modulus of the gel mixtures while the variation of P407 concentration (2.5%-10%) minimally changed the composite moduli. The gel mixture displayed thermo-softening behavior although P407 itself is thermo-setting and the initial melting temperature of the gel mixture (approximately 55ºC) was governed by P407. Although the copolymer gels are regularly used in drug-delivery systems[31], the bioactive agents (like viruses and proteins) may not be stable at high temperatures. Thus, the composite should be further optimized for its applications in drug delivery. In addition, these agarose/P407 gel mixtures exhibited shear thinning behavior similar to agarose gel, which may indicate that they are suitable for injection applications. For example, this composite can be injected to deep tissues to repair articular cartilage defects or fill the irregular spinal cord defects[32, 33]. It can also be adopted as nerve guidance scaffolds to enhance tissue regeneration[34]. However, full regeneration may not be achieved due to the non-degradability of the mixture. In the future, more replicates in mechanical characterization should be done to better reveal the physics of these hydrogels.

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